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Hemoglobins in Togolese Newborns: Hb S, Hb C, Hb Bart's, and α-Globin Gene Status

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The gene frequency of the most important hemoglobin (Hb) abnormalities is reported in a population of 171 Togolese newborns. Hb phenotypes, hematological parameters, and the more frequently described α-gene deletions were analyzed. Structural abnormalities of β-globin were observed in 35.7% of the children with a gene frequency of 0.105 for β^S and 0.091 for β^C. The frequency of the different α-globin genotypes was αα/ = 0.71, −α/ = 0.28, and ααα/ = 0.01. All of the individuals homozygous for the −α/ genotypes, and most of the heterozygous individuals, carried Hb Bart's. Within the αα/αα and the −α/αα groups, several individuals with or without Hb Bart's were found; they did not differ from the others by their red blood cell (RBC) parameters but by their levels of fetal hemoglobin (Hb F). The African α2 polymorphism marker, characterized by the replacement of G by TCGGCCC at position 7238 (EMBL HSHBA4, 1993) and of T 7174 by G, was found in 21 newborns. The mean value of Hb F was calculated for each genotype, the mean $^G\gamma$ percentage was 69.4 ± 4.0%, and the gene frequency of the $^A\gamma^T$marker was 0.10; this marker was linked to the normal β-globin cluster. Am. J. Hematol. 59:208–213, 1998.

Key words: Hb Bart's; Hb F; α-globin genotypes; Togo

INTRODUCTION

In West Africa, many hemoglobin (Hb) disorders, involving both the α- and β-globin chains, occur in various frequencies. The two most common are Hb S and Hb C. In a survey done by Cabannes et al. in 1987 [1], the frequencies of β-thalassemia and of α-thalassemia were found to vary from 0.5 to 7% and from 0.2 to 2.2%, respectively, according to the location of the study. These Hb disorders may be protective factors against *Plasmodium falciparum* [2]. In addition, α-thalassemia could be of benefit in the course of sickle cell anemia [3]. In Togo, as in the other African Countries, Hb disorders constitute a major problem for public health [4,5].

In the Togolese population, previous studies reported that the frequency of Hb abnormalities was 37.6% in newborns [5] and 34.1% in a group of rheumatology patients [6]. The frequencies of S and C genes were 0.110 and 0.053, respectively [5]. In this work, we report the gene frequencies of the most important Hb abnormalities in a population of 171 Togolese newborns. The Hb phenotypes, hematological parameters, and the more frequently described α-gene deletions were analyzed. To find out whether an accurate determination of Hb Bart's at birth would detect all the cases of deletional α-thalassemia, a comparison was done between the α-gene status and the Hb Bart's phenotype of all the children. In addition, the presence of the African α2 polymorphism, previously described by Préhu and colleagues [7], was checked in all the newborns.

MATERIALS AND METHODS

Preparation of the Samples

One hundred seventy-four cord blood samples were collected without selective criteria on ethylenediamine-

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tetraacetic acid (EDTA) from children born during a three-week period in the three maternity hospitals in Lomé (Togo). Three samples were excluded from the study because of the poor quality of their DNA, which did not allow molecular examination. Red blood cells (RBCs) were washed three times in NaCl (9%) and stored at −70°C. Packed white blood cells were collected by selective hemolysis of the blood pellet using EDTA (25 mM, pH 8.0) − TRIS (20 mM, pH 7.5) and stored at −70°C.

Hematological Analysis

RBC counts were performed on an automated Coulter Counter (Coulter T 540) (Beckman Coulter, Fullerton, CA) providing eight parameters: red cell, white cell, and platelet counts, Hb level, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Hb Phenotype

Isoelectric focusing on thin-layer agarose gel was performed according to Hocking [8]. Hb F and A_2 were assayed using the β-Thalassemia Short Program on a Biorad Variant Hemoglobin Testing system (Bio-Rad Labs, Hercules, CA). A modification in the starting time of the integration program allowed us to measure Hb Bart's. The threshold value for detection of Hb Bart's was 0.5%.

Fetal Hemaglobin Subunit Composition

Fetal Hemoglobin (Hb F) subunit composition ($^{G}\gamma$, $^{A}\gamma$, $^{A}\gamma^{T}$) was obtained by reverse-phase perfusion chromatography using the Poros R1 column (PerSeptive Biosystems, Cambridge, MA) [9]. This procedure involves a high velocity flow of the mobile phase through porous chromatographic particles (10 μm diameter) made of polystyrene-divinylbenzene. Elution was obtained at a flow rate of three ml/min by developing a gradient from 36 to 41% acetonitrile. This measurement was done in all the newborns.

Molecular Biology Studies

DNA was prepared from packed white cells by standard phenol-chloroform extraction. The −3.7 and −4.2 kb deletions of the α genes were investigated by polymerase chain reaction (PCR) analysis on a Perkin Elmer thermocycler (Norwalk, CT) according to Dodé et al. [10] with the following modification: denaturation at 94°C for 5 min in the first cycle; annealing 1.5 min at 57°C, extension at 72°C for two min in each of the 30 subsequent cycles; and final elongation at 72°C for seven min. Three primers were used for characterization of the $\alpha^{-3.7}$ deletion: the upstream oligonucleotide C10 [10], and the α1 and α2 specific downstream primers C2 and C3 [11]. For determination of the $\alpha^{-4.2}$ deletion, three primers were also used: the upstream oligonucleotide (5′ TAGGACAGACCCTCCCACGCGTAAG 3′) which is located within IVS2 of Ψα1 gene; the downstream primers (5′TGTCCAGGAGCAAGCCAGGGTAAG 3′) between α1 and α2; and (5′ TGCACGCCTCCCTGGACAAGTT 3′) located in exon 3 of the α1 gene. The experimental conditions for amplification were identical to those described above. PCR products were analyzed by electrophoresis on a 6% polyacrylamide gel.

The African α2 polymorphism was determined as described previously; this abnormality is observed as heteroduplexes when the PCR products containing the α2 gene, selectively amplified according to the procedure of Dodé and colleagues [10], are analyzed by electrophoresis in a 6% polyacrylamide gel.

Statistical Analysis

Comparisons between α-thalassemia genotypes were made using the χ^2 test. For the comparison of quantitative traits, analysis of variance was used.

RESULTS

Description of the Population

Ninety-three female and 78 male cord blood samples were analyzed. Seven children were premature. In Togolese newborns the mean values for Hb, MCV, and MCH were 13.1 ± 2.1 g/dl, 106.1 ± 7.4 fl, and 34.5 ± 3.3 pg, respectively.

Hb Variants

Hb electrophoretic studies revealed that two children were homozygous for sickle cell disease (as controlled by restriction endonuclease *Bsu* 36 I), four were compound heterozygotes for Hb S and Hb C, 28 were heterozygotes for Hb S, and 27 for Hb C. In 110 newborns the electrophoretic pattern showed only Hb A as adult Hb. At least one child could be suspected, from his hematological data (Hb = 10.2 g/dl, MCV = 97.8 fl, MCH = 27.2 pg), and from the increased Hb A_2 and Hb F levels of his mother, to be a carrier of a β-thalassemia trait, but this has not been verified by molecular biology studies. Altogether, structural abnormalities of β-globin were observed in 35.7% of the newborns. The calculated Hb gene frequency was 0.105 for β^{S} and 0.091 for β^{C}.

Hb F

Hb F composition revealed a $^{G}\gamma$ percentage of 69.4 ± 4.0%, except in one case in which it was 41%. Hb F Sardinia ($^{A}\gamma^{T}$ marker) was found in the heterozygous form in 28 newborns and in the homozygous form in three of them, which had only Hb A. The heterozygous form of F Sardinia was observed in 20% of the newborns carrying only Hb A and in 10.9% of those which were

TABLE I. α Gene Status and Hb Phenotype*

	αα/αα		−α/αα		−α/−α		ααα/ααα	
	N	M/F	N	M/F	N	M/F	N	M/F
AA (n = 110)	49	22/27	51	24/27	8	5/3	2	1/1
AS (n = 28)	20	11/9	5	3/2	3	2/1	0	—
AC[a] (n = 27)	15	7/8	7	0/7	2	0/2	2	1/1
SC (n = 4)	2	1/1	2	0/2	0	—	0	—
SS (n = 2)	0	—	1	1/0	1	0/1	0	—

*N, number, M, males, F, females.
[a]Not shown, one case with an atypical α-gene electrophoretic pattern.

TABLE II. RBC Parameters According to the α-Globin Gene Status[†]

	αα/αα (n = 86)	−α/αα (n = 66)	−α/−α (n = 14)
Hb (g/dl)*	13.6 ± 1.8	12.6 ± 2.7	12.7 ± 0.9
MCV (fl)**	109.8 ± 6.0	102.8 ± 7.8	92.2 ± 5.8
MCH (pg)**	35.8 ± 2.2	33.3 ± 3.2	27.9 ± 1.8
MCH_F (pg)**	29.8 ± 3.6	25.9 ± 4.5	20.5 ± 2.4
Hb F (%)**	83.1 ± 7.6	76.0 ± 13.0	73.9 ± 9.4
Hb F + Bart's (%)*	83.5 ± 7.8	79.7 ± 9.6	81.3 ± 8.3

[†]Not shown, four cases of α-triplication and one case with an atypical α-gene electrophoretic pattern. Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; Hb F, fetal hemoglobin.
*$P < 0.05$.
**$P < 0.001$.

heterozygous for Hb S and Hb C. This suggests that $^{A}\gamma^{T}$ is linked to the Hb A chromosome except for one SC newborn. The calculated gene frequency of $^{A}\gamma^{T}$ marker was 0.10. The Hb F level and percentage of Hb Bart's were identical in individuals with or without the $^{A}\gamma^{T}$ marker.

α-Globin Genes Status

Sixty-six newborns were heterozygous for the $\alpha^{-3.7}$ deletion, 14 were homozygous, and four had an α-gene triplication. The frequency of the different genotypes was αα/ = 0.71, −α/ = 0.28, and ααα/ = 0.01. The distribution of the α-globin gene status was statistically identical among the various β-chain Hb phenotypes (Table I).

The hematological data corresponding to the various α-globin gene status are given in Table II. They show the expected difference of the various RBC parameters in relation to the number of delected α genes.

Hb Bart's Phenotypes

Hb Bart's was observed in 84 newborns: 58 of those carried only Hb A, 14 were heterozygous AS, and eight were heterozygous AC. Hb Bart's was found in two of the four compound heterozygotes SC and in the two homozygotes SS. The frequency was the lowest in the AC group. Hb Bart's levels ranged from our threshold value of 0.5 to 17%.

Amont the children with four α genes, two groups could be distinguished on the basis of the presence or absence of Hb Bart's: a major one (n = 71) in which no Hb Bart's was detectable; and a minor one (n = 15) with an Hb Bart's level of 2.1 ± 1.6%. As shown in Table III the RBC parameters between these two groups are identical with the exception of those concerning Hb F. The Hb F level was 82.2 ± 7.8% in the first group and 87.3 ± 4.9% in the second one; the MCH_F was 29.32 ± 3.42 pg vs. 32.03 ± 3.46 pg. We also noticed a significant difference of weight at birth between these two groups. A similar situation was observed among the newborns with the −α/αα genotype: in this case the main group (n = 56) had Hb Bart's with a mean value of 2.5 ± 2.1%, while in a smaller group (n = 10) there was no detectable Hb Bart's. Again the RBC parameters were identical between both groups with the exception of the Hb F level,

TABLE III. Differences Between αα/αα and −α/αα Newborns According to the Presence of Hb Bart's*

	αα/αα		−α/αα	
	Bart's− n = 71	Bart's+ n = 15	Bart's− n = 10	Bart's+ n = 56
Hb Bart's (%)	0 ± 0	2.1 ± 1.6	0 ± 0	2.6 ± 2.1
Hb (g/dl)	13.7 ± 1.9	12.8 ± 1.6	12.6 ± 2.3	12.7 ± 2.6
MCV (fl)	109.7 ± 6.0	110.6 ± 6.1	102.0 ± 4.8	103.7 ± 4.3
MCH (pg)	35.7 ± 2.2	36.6 ± 2.2	33.4 ± 3.2	33.6 ± 2.9
MCH_F (pg)	29.3 ± 3.4	32.0 ± 3.5	23.2 ± 3.9	26.6 ± 4.2
Hb F (%)	82.2 ± 7.7	87.3 ± 4.9	69.1 ± 8.6	79.0 ± 8.8
Hb F + Bart's (%)	82.2 ± 7.7	89.5 ± 4.5	69.1 ± 8.6	81.6 ± 8.6
Weight (g)	2864.1 ± 419.8	2422.7 ± 660.4	2866 ± 372.1	2822.3 ± 538.5

*See Table II for abbreviations. MCH_F: $P < 0.01$ between the two αα/αα groups; $P < 0.05$ between the two −α/αα groups. Body weight: $P < 0.001$ between the two αα/αα groups; NS between the two −α/αα groups.

TABLE IV. RBC Parameters in Newborns Carrying the African Polymorphism Marker*

	αα/αα (n = 14)	−α/αα (n = 6)
Hb (g/dl)	13.2 ± 2.0	12.0 ± 1.7
MCV (fl)	110.4 ± 3.4	106.2 ± 4.1
MCH (pg)	36.6 ± 1.5	34.1 ± 1.6

*See Table II for abbreviations. Not included, a case of α-gene triplication associated to the marker. For comparison see values in Table II.

79.0 ± 8.8% in the first group and 69.1 ± 8.6% in the second one, and the MCH_F. By contrast, Hb Bart's was observed in all the newborns with the −α/−α genotype; its level was clearly higher (7.32 ± 3.48%).

In the individuals with Hb Bart's who did not have the $\alpha^{-3.7}$ deletion, the $\alpha^{-4.2}$ deletion was looked for and never found. The absence of this deletion was also verified in the few newborns who had one or two α genes deleted and the highest level of Hb Bart's for their group.

African α2 Polymorphism Marker

The African α2 polymorphism marker, characterized by the replacement of G with TCGGCCC at position 7238 (EMBL HSHBA4, 1993) and of T 7174 by G, was found in 21 newborns. Twenty were heterozygous for this marker, and one was homozygous. Six heterozygotes were associated with a −3.7 Kb deletion and one with an α-gene triplication. Table IV shows that this polymorphism was without effect on the RBC parameters in the newborns classifed according to their α-globin gene status.

DISCUSSION

Detection of Hb abnormalities at birth offers many advantages. Collection of cord blood samples is not traumatic, easy to practice, and allows systematic investigation of a large number of individuals. In African populations, the particular interest of such studies is to detect sickle cell syndromes as early as possible to prevent complications. Under these conditions it is also possible to detect Hb Bart's that has been considered as a marker of α-thalassemia.

In our study, the Hb level was significantly lower from that found in Caucasian newborns, which is 13.1 ± 2.1 vs. 16.5 ± 3.0 g/dl [12] likely related to environmental factors. We found that the frequency of Hb abnormalities was 35.7% with an almost identical predominance of Hb S and Hb C (Table I). Two children were found homozygous for Hb S and four were compound heterozygotes for Hb S and Hb C. These data agree with those reported by Mijiyawa and colleagues [6], North and colleagues [5] in Togo, and with those of Cabannes and colleagues in the Ivory Coast [1], as shown in Table V.

The α-thalassemias are the most common single gene disorders in humans. They are most frequently due to deletions involving one (−α/) or both (−−/) of the α-globin genes that map to the terminal band of the short arm of chromosome 16 [13,14]. In Africa, only the −α has been found. It has been proposed that α-thalassemias may be suspected by the presence of Hb Bart's at birth. We observed a high proportion of Togolese newborns carrying Hb Bart's (49.2%). This value was much higher than that reported previously: 6.7% in the studies of North and colleagues in Togo [5]; and 4.3% in that of Sangaré and colleagues in the Ivory Coast [15]. This difference may be explained by the technical progress in measuring small amounts of Hb Bart's. According to the number of α genes, we found three categories of newborns carrying Hb Bart's (Fig. 1). In the first group, homozygous for the $\alpha^{-3.7}$ deletion, all the children but one had a high level of Hb Bart's (7.3 ± 3.5%). One of these children displayed an Hb Bart's level of 16.9%; the reason for this is under investigation. In the group of newborns heterozygous for the deletion, Hb Bart's was observed in 85% of the cases, the level of Hb Bart's was about 2% and the mean Hb F level was 79.0 ± 8.8%. In the 15% of this group in which Hb Bart's was not detected, the mean Hb F level was 69.1 ± 8.6%. Eighty-three percent of the children without $\alpha^{-3.7}$ deletion were

TABLE V. Hemoglobin Abnormalities Frequency in Sub-Saharan Africa

	AA (%)	AS (%)	AC (%)	SC (%)	SS (%)	CC (%)
Ivory Coast Cabannes and colleagues (1987) (newborns, n = 4,000)	79.7	8.7	6.6	0.4	0.6	0.4
Togo North and colleagues (1988) (newborns, n = 385)	63.3	18.7	8.9	1.3	1	0.3
Togo Mijiyawa and colleagues (1994) (rheumatology patients, n = 405)	64.9	15.8	12.1	4.2	2	0.5
Togo Our study (1997) (newborns, n = 171)	64.3	16.4	15.8	2.3	1.1	0

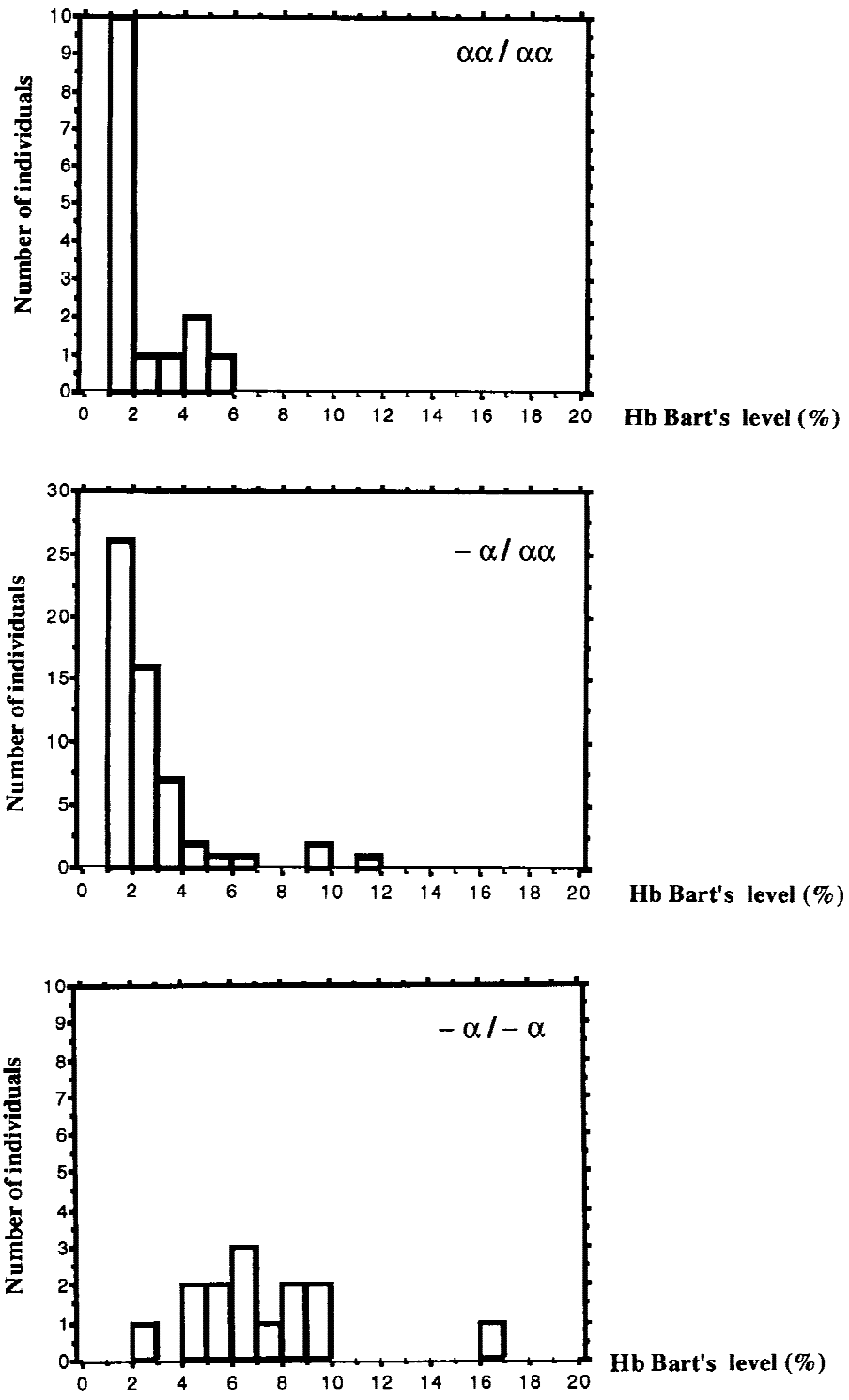

Fig. 1. Distribution of Hb Bart's among children with αα/αα, −α/αα and −α/−α genotypes.

free of Hb Bart's; their mean Hb F level was 82.2%. In the 17% remaining, in which the mean Hb F value was 87 ± 4.9%, trace amounts of Hb Bart's were detected and, in addition, these children had a lower weight at birth. The presence of Hb Bart's seems therefore to depend on both the number of active α genes and on the level of fetal Hb. The hematological data of the different groups of newborns are also shown in Table II. It must be noted that the MCV and MCH decrease in a similar way in the children with or without Hb Bart's, proportional to the number of α genes deleted. Within the αα/αα and the −α/αα groups, the RBC parameters were not different between individuals with or without Hb Bart's and we found that the only difference was the level of Hb F. In

the absence or in the presence of low amounts of Hb Bart's, the level of Hb F and the RBC parameters are significantly different, allowing distinction between the $-\alpha/\alpha\alpha$ and $\alpha\alpha/\alpha\alpha$ genotypes. Our results suggest therefore that previous studies on α-thalassemia epidemiology, done solely by measurement of Hb BArt's at birth and RBC parameters, should be considered with caution.

The mean $^{G}\gamma$ percentage observed in our study was similar to that reported by Wajcman et al. [16] during a systematic study done in Paris in a program of neonatal screening for sickle cell disease. In one case, it amounted to 41%, which could be explained by a γ-gene rearrangement [17]. $^{A}\gamma^{T}$ marker was found with a frequency of 18.7%, which is also similar to that of the previous studies [16,18]. This marker was always found associated with the A chromosome except for an SC newborn. Newborns with the S Cameroon haplotype were not found in this Togolese population.

African polymorphism marker of the α2-globin gene was found in the Togolese newborns with a frequency similar to that reported by Préhu and colleagues [17] in a study performed on 306 sickle cell disease patients from African followed in the sickle cell center in Paris. The results of Hb Bart's, MCV, and MCH showed no significant difference between newborns positive or negative for the presence of this marker, confirming that it has no α-thalassemia effect. This marker seems to be associated with the normal αα chromosomes.

In conclusion, our study confirmed the high frequency of Hb S and Hb C and revealed a high frequency of the $\alpha^{-3.7}$ deletion. About 3% of the newborns carry a sickle cell disease. A good correlation between the RBC parameters and the α-gene status was demonstrated, but frequently individual cases do not correlate with the presence of Hb Bart's; the influence of Hb F on the Hb phenotype has to be emphasized. Thus, in cases with high Hb Barts's and low Hb F, an additional α-gene abnormality may be suspected.

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